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RESEARCH ARTICLE

Chronic ciprofloxacin and atrazine co-exposure aggravates locomotor and exploratory deficits in non-target detritivore speckled cockroach (*Nauphoeta cinerea*)

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Abstract

The global detection of ciprofloxacin and atrazine in soil is linked to intensive anthropogenic activities in agriculture and inadvertent discharge of industrial wastes to the environment. *Nauphoeta cinerea* is a terrestrial insect with cosmopolitan distribution and great environmental function. The current study probed the neurobehavioral and cellular responses of *N. cinerea* singly and jointly exposed to atrazine (1.0 and 0.5 μg g^{-1} feed) and ciprofloxacin (0.5 and 0.25 μg g^{-1} feed) for 63 days. Results demonstrated that the reductions in the body rotation, maximum speed, turn angle, path efficiency, distance traveled, episodes, and time of mobility induced by atrazine or ciprofloxacin per se was exacerbated in the co-exposure group. The altered exploratory and locomotor in insects singly and jointly exposed to ciprofloxacin and atrazine were verified by track plots and heat maps. Furthermore, we observed a decrease in acetylcholinesterase and anti-oxidative enzyme activities with concomitant elevation in the levels of lipid peroxidation, nitric oxide, and reactive oxygen and nitrogen species were significantly intensified in the midgut, hemolymph, and head of insects co-exposed to ciprofloxacin and atrazine. In conclusion, exposure to binary mixtures of ciprofloxacin and atrazine elicited greater locomotor and exploratory deficits than upon exposure to the individual compound by inhibiting acetylcholinesterase activity and induction of oxido-inflammatory stress responses in the insects. *N. cinerea* may be a usable model insect for checking contaminants of ecological risks.

Keywords Anthropogenic activities · Chemical mixtures · Insects · Neurobehavior · Acetylcholinesterase · Oxido-inflammatory stress

Introduction

Antibiotics are antimicrobial drugs widely used in human and veterinary medicine for the prevention and treatment of bacterial infections. They are globally acknowledged as pollutants, leading to major public concern due to their incomplete removal by wastewater treatment plants resulting in their release to the ecological environments (Liu and Wong 2013). Numerous antibiotics including fluoroquinolones have been identified in sewage sludge frequently applied to amend soil (Bondarczuk et al. 2016). Ciprofloxacin, a broad-spectrum fluoroquinolone antibiotic, is normally prescribed to treat infections caused by both gram-negative and gram-positive bacteria in humans and animals (Sisay et al. 2018). Large quantities of ciprofloxacin are often detected in urine and feces of humans and animals owing to its incomplete metabolism. The maximum concentrations of ciprofloxacin in sewage sludge ranges from 1600 to 11,000 μg/kg (Östman et al. 2017) and from 9.4 to 126.3 μg/g in the soil samples (Li et al. 2015; Arun et al. 2020). The soil is often directly contaminated with ciprofloxacin by the release of waste from farm animals (Wei et al. 2019). Moreover, the direct application of manure

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fertilizers is another means by which the soil enriched is by ciprofloxacin (Zhang et al. 2016).

Previous ecotoxicological studies evidenced that time-dependent exposure of earthworms (*Eisenia fetida*) to ciprofloxacin at 10 mg/kg resulted in a significant disruption of antioxidant enzyme activities and induction of DNA damage (Yang et al. 2020). Oral administration of rats with ciprofloxacin at 20 and 50 mg/kg for 14 days induced neurotoxicity by decreasing the levels of brain neurotransmitter and antioxidant status with concomitant increase in oxidative stress (Ilgin et al. 2015). Moreover, sub-lethal concentrations (0.95, 1.9, and 3.2 mg/mL) of ciprofloxacin adversely induced developmental, physiological, physical, and biochemical changes in *Drosophila melanogaster* (Liu et al. 2019).

Atrazine is an herbicide extensively used across the globe owing to its high effectiveness in controlling weeds and low cost (Griboff et al. 2014). It is a contaminant of emerging concern due to its ubiquity, mobility, and persistence in the environment where non-target organisms are adversely impacted (Cheng et al. 2020). The maximum concentration of atrazine in polluted soil ranges from 0.134 to 1100 mg/kg and from 0.73 to 81.3 μg/L in groundwater (Komtchou et al. 2017; Dou et al. 2020). Earlier studies demonstrated that atrazine elicits detrimental effects on terrestrial and aquatic ecosystems (Song et al. 2009; Zhu et al. 2011). Exposure of *Xenopus laevis* to atrazine at environmentally relevant concentrations caused significant functional alterations in cardiac tissue contractility, with ensuing mortality in tadpoles (Asouzu Johnson et al. 2019). Moreover, atrazine adversely affected immunity in dragonfly, *Pachydiplax longipennis*, by modulating hemocyte numbers and phenyloxidase activity in the cuticule, gut, and hemolymph (St Clair and Fuller 2018). Acute exposure to atrazine elicited low motor activity and adversely impacted biochemical profile of hemolymph, antioxidant status, and hepatopancreas histology in crayfish *Cherax destructor* (Stara et al. 2018). Moreover, atrazine has been documented to disrupt endocrine function, induced reproduction, and developmental deficit as well as DNA damage in crayfish, fish, and mammals (Saalfeld et al. 2018; Abdulelah et al. 2020). The noxious effects of atrazine on *Caenorhabditis elegans* were palpable on the fertility, locomotion, and survival in a dose-dependent manner (García-Espiñeira et al. 2018). The percentages of pupated and emerged flies as well as the survival of adult flies were decreased in *Drosophila melanogaster* exposed to atrazine (Marcus and Fiumera 2016). Numerous ecotoxicological investigations have also demonstrated that atrazine induces ROS production and oxidative stress in exposed organisms (Semren et al. 2018; Wang et al. 2019; Blahova et al. 2020).

Atrazine and ciprofloxacin detection in soil is associated with intensive anthropogenic activities in agriculture and inadvertent discharge of industrial wastes to the environment. Terrestrial organisms are commonly exposed to toxic substances in the soil which largely acts as a sink for complex mixtures of contaminants (Adedara et al. 2020a). Cockroaches are terrestrial insects with cosmopolitan distribution and high environmental function (Bell et al. 2007; Adedara et al. 2020a). They are found mostly in tropical and damp environments, where they feed on debris and, as a result, contribute to recycling of nutrient in the soil. Cockroaches ingest decomposed organic substances and discharge nitrogen into the environment through their feces. Additionally, cockroaches are good nutritional source for a number of insectivores, namely reptiles, birds, and mammals, consequently, forming a major component of the food web in bionetwork.

Cockroaches are widely used insect models in ecotoxicology research for the evaluation of the effects of contaminants. Apart from the relative simplicity in the nervous system in insects and vertebrates (Blankenburg et al. 2015; Stankiewicz et al. 2012), insects have a lateralized organization of their brains and behavior, analogous to vertebrates. This brain organization affects several behavioral ecology contexts that are crucial for the fitness of a species (Bell and Niven 2016; Romano et al. 2020). Cockroaches are easier to maintain, smaller to handle, and very prolific model organism (Harris and Moore 2005). Moreover, cockroaches are a good insect model for long-term studies, because female and male cockroaches can live for 344 and 365 days, respectively. Earlier ecotoxicological investigations using cockroaches revealed that environmental contaminants, namely mercury, 4-vinylcyclohexane, and fluoranthene, induced oxidative stress in the insect (Rodrigues et al. 2013; Waczuk et al. 2019; Mrdaković et al. 2019). Furthermore, cockroaches have been used as model insects for the assessment of neurotoxicity linked to organophosphates trichlorfon (Stürmer et al. 2014), chlorpyrifos (Adedara et al. 2016), methyl mercury (Afolabi et al. 2020; Adedara et al. 2015), analgesic, and psychoactive drugs (Adedara et al. 2020a, 2021).

Despite the fact that ciprofloxacin and atrazine often coexist in mixtures, information concerning their impact on terrestrial insects is lacking. Toxicological outcome of chemical mixtures may elicit a stronger consequence (that is, synergistic or additive) or a feebler consequence (that is, inhibition or antagonism (Groten et al. 2001; Adedara et al. 2017). Thus, in view of the potential pervasiveness of ciprofloxacin and atrazine in the environment, it is important to deepen the extant knowledge about the toxicological responses of insects to these anthropogenic contaminants. The head of a cockroach includes the brain which coordinates the body functions including behavior whereas the midgut is primarily responsible for digestion and uptake of nutrient. The hemolymph is an arthropod's circulatory fluid which is analogous to the blood in vertebrates (Adedara et al. 2020a). The current investigation evaluated, for the first time, changes in behavioral characteristics, antioxidant enzyme activities, and oxido-inflammatory stress in the midgut, head, and hemolymph of non-target *N. cinerea* nymphs singly or jointly exposed to ciprofloxacin and atrazine.

Materials and methods

Chemicals

Technical grade atrazine procured from Shandong Vicome Greenland Chemicals Company Limited, Shandong, China, was used for the current investigation. Ciprofloxacin, 2′,7′-dichlorofluorescein diacetate (DCFH-DA), bovine serum albumin (BSA), acetylthiocholine iodide, hydrogen peroxide (H_2O_2), 1-chloro-2,4-dinitrobenzene (CDNB), sodium azide (NaN_3), glutathione (GSH), sulphosalicylic acid, thiobarbituric acid (TBA), trichloroacetic acid (TCA), and 5′,5′-dithiobis-2-nitrobenzoic acid (DTNB) were gotten from Sigma Aldrich (St. Louis, MO, USA).

Nurturing of insects' nymphs and diet preparation

The *N. cinerea* nymphs used for the current investigation were collected from the insect colony in the Department of Biochemistry, University of Ibadan. The insects were maintained under normal laboratory conditions of 22–24 °C room temperature, 60–70% relative humidity, and photoperiod of 12-h light/dark cycle with unrestricted consumption of water and insect diet (Adedara et al. 2015). Atrazine and ciprofloxacin were individually dissolved in absolute ethanol before adding to dry diet. A total of 300 mg of contaminated and normal food were individually added to 180 μL of ethanol (1.0% final concentration). Normal diets, those contaminated with atrazine or ciprofloxacin alone as well as their binary mixtures, were left in the open air until the ethanol was fully evaporated. The diets were thereafter stowed at − 20 °C.

Treatment of insects

The experimental design comprised of six groups of fifteen *N. cinerea* nymphs each. The insects were fed ad libitum with food containing the test compounds for 63 consecutive days (9 weeks) in this manner.

Control: Insects fed with normal feed.
ATZ1.0 alone: Insects fed with food containing atrazine (ATZ) alone at 1.0 μg g^{-1} diet.
CPFX0.5 alone: Insects fed with food containing ciprofloxacin (CPFX) alone at 0.5 μg g^{-1} diet.
ATZ0.5 + CPFX0.25: Insects that ate food contaminated with ATZ at 0.5 μg g^{-1} diet and CPFX at 0.25 μg g^{-1} diet.
ATZ0.5 + CPFX0.5: Insects that ate food contaminated with ATZ at 0.5 μg g^{-1} diet and CPFX at 0.5 μg g^{-1} diet.
ATZ1.0 + CPFX0.5: Insects that ate food contaminated with ATZ at 1.0 μg g^{-1} diet and CPFX at 0.5 μg g^{-1} diet.

The exposure period and concentrations of atrazine and ciprofloxacin were obtained from the preliminary studies to identify the sub-lethal, environmentally relevant concentrations that would disrupt behavioral patterns of the cockroaches.

Assessment of neurobehavioral characteristics

The changes in behavior of the insects after exposure period were assessed in a novel apparatus as previously described (Adedara et al. 2015). Briefly, 15 insects (same age and both sexes) from each group were carefully taken, one at a time, and placed in the new apparatus to avoid handling distress during the investigation. Acclimatization period of about 1 min was noted before starting the test. The behavioral assessment which lasted 8 min was filmed using a DNE webcam (Porto Alegre, Brazil) sited directly overhead the apparatus and joined to a laptop computer throughout the trial. The behavioral parameters were automatically generated by suitable video-tracking software (ANY-maze, Stoelting CO, USA). Specifically, exploratory activities in the horizontal and vertical parts of the apparatus were appraised by the heat maps and track plots created using the software. Locomotion indices including maximum speed, mobile episodes, path efficiency, distance traveled, freezing time, turn angle, and body rotation of the insects were appraised.

Preparation of samples for biochemical endpoints

Biochemical analyses were carried out subsequent to behavioral evaluation. Briefly, all the 15 insects from each group were separately anesthetized with ice and weighed before careful removal of the hemolymph, midgut, and head into Eppendorf tubes. Subsequently, homogenization of the samples was done separately using ice-cold 0.1-M phosphate buffer, pH 7.4 in ratio of 1:35 (mg:μL buffer), whereas centrifugation of the homogenates was done at 6000*g* for 10 min at 4 °C to obtain the supernatant for the biochemical endpoints. The concentration of the protein in the hemolymph, midgut, and head was determined at 595 nm, as previously stated (Bradford 1976).

Assay of neurotoxicity and oxido-inflammatory stress biomarkers

Activity of acetylcholinesterase (AChE), an indicator of nervous system function, was assayed in the head supernatants at a wavelength of 412 nm as earlier stated (Ellman et al. 1961). Indices of oxidative stress and antioxidant status were appraised in the hemolymph, midgut, and head using established methods. Level of malondialdehyde (MDA), an index of lipid peroxidation (LPO), was analyzed at 532 nm as earlier stated (Adedara et al. 2015) whereas reactive oxygen and nitrogen species (RONS) levels was assessed at 488 nm (excitation) and 525 nm (emission) using standard protocol (Adedara et al. 2016). Levels of glutathione (GSH) were assayed at 412 nm according to established protocol (Jollow et al. 1974). Catalase (CAT) activity was determined at 240 nm (Aebi 1984), superoxide dismutase (SOD) activity was analyzed at 480 nm (Misra and Fridovich 1972), and glutathione-S-transferase (GST) and glutathione peroxidase (GPx) activities were determined at 340 nm and 412 nm, respectively, as previously stated (Habig et al. 1974; Rotruck et al. 1973). Furthermore, the amount of nitric oxide (NO), an inflammatory biomarker, was analyzed at 540 nm in the supernatants using established procedure (Green et al. 1982). Activities of CAT and SOD were analyzed with 752S UV-VIS Spectrophotometer (Ningbo, China), while the remaining biochemical assays were carried out with a SpectraMax plate reader (Molecular Devices, CA, USA).

Statistical analyses

Normal distribution and homogeneity of the data were verified by Kolmogorov-Smirnov and Bartlett's tests, correspondingly. The data were analyzed using one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparisons test with the aid of GRAPHPAD PRISM 5 software (Version 4; GraphPad Software, La Jolla, CA, USA). Statistical significance was assigned at $p < 0.05$.

Results

Co-exposure to atrazine and ciprofloxacin aggravates locomotor and motor deficits in *N. cinerea*

Separate and joint exposure to atrazine and ciprofloxacin did not significantly affect consumption of food and survival rate of the exposed insects compared with control (data not shown). Locomotor and motor activities of insects exposed to atrazine or ciprofloxacin alone and their binary mixtures in the new environment are shown in Fig. 1. Locomotor parameters specifically distance traveled ($F_{5,89} = 140.8$; $p < 0.0001$), episodes of mobility ($F_{5,89} = 46.46$; $p < 0.0001$), time mobile ($F_{5,89} = 56.35$; $p < 0.0001$), and maximum speed ($F_{5,89} = 94.60$; $p < 0.0001$) were markedly reduced in insects exposed to atrazine alone or ciprofloxacin alone in comparison to control group. In addition, motor and turning activities precisely the body rotation ($F_{5,89} = 85.81$; $p < 0.0001$), ability to travel in a straight path ($F_{5,89} = 65.03$; $p < 0.0001$), and turn angle ($F_{5,89} = 98.60$; $p < 0.0001$) were significantly decreased in insects separately exposed to atrazine or ciprofloxacin in comparison with the control. The marked reductions in the locomotor parameters as well as motor and turning activities were considerably exacerbated in the insects jointly exposed to the binary mixtures of atrazine and ciprofloxacin. The marked elevation in the freezing time ($F_{5,89} = 164.6$; $p < 0.0001$), reflecting an anxiogenic marker, was exaggerated in cockroaches fed with feed-containing binary mixtures of atrazine and ciprofloxacin compared to control.

Co-exposure to atrazine and ciprofloxacin exacerbates horizontal and vertical exploratory actions in *N. cinerea*

As verified by the data from exploratory behavior analyses presented in Figs. 2 and 3, separate exposure of insects to atrazine and ciprofloxacin significantly increased the latency to first entry to the vertical zone ($F_{5,89} = 61.64$; $p < 0.0001$), but significantly diminished the latency to first entry to the horizontal zone ($F_{5,89} = 64.36$; $p < 0.0001$) in the novel apparatus, compared to control. Binary mixtures of atrazine and ciprofloxacin exacerbated the latency of insects' first entries to both zones of the new apparatus. Moreover, separate treatment with atrazine and ciprofloxacin considerably decreased the total time spent ($F_{5,89} = 134.7$; $p < 0.0001$) and mean visit ($F_{5,89} = 310.5$; $p < 0.0001$) to vertical zone, but significantly increased the total time spent ($F_{5,89} = 169.6$; $p < 0.0001$) and mean visit ($F_{5,89} = 493.1$; $p < 0.0001$) in the horizontal zone compared to control. The impact of exposure on the mean visit and time spent in the vertical and horizontal zones was exacerbated in cockroaches exposed to the binary mixtures compared to control and individual exposures. Exposure of insects to mixtures of atrazine and ciprofloxacin intensified the time of freezing in both vertical ($F_{5,89} = 64.40$; $p < 0.0001$) and horizontal ($F_{5,89} = 130.9$; $p < 0.0001$) zones compared with individual exposure and control. The interference of individual and joint exposures to atrazine and ciprofloxacin on the exploratory activity of insects was further verified by the track plots, which revealed their wandering traces, and heat maps. The greater heat map intensity and decline in the track plots density in insects separately treated with atrazine and ciprofloxacin were aggravated following exposure to their binary mixtures.

Atrazine and ciprofloxacin co-exposure impairs activity of AChE and antioxidant defense mechanism in *N. cinerea*

Effects of exposures to separate and binary mixtures of atrazine and ciprofloxacin on the activity of AChE and antioxidant defense system specifically GSH, CAT, SOD, GST, and GPx in exposed and control insects are presented in Figs. 4, 5, and 6. The AChE activity ($F_{5,89} = 72.45$; $p < 0.0001$) in the head and GSH level in the midgut ($F_{5,89} = 52.20$; $p < 0.0001$), hemolymph

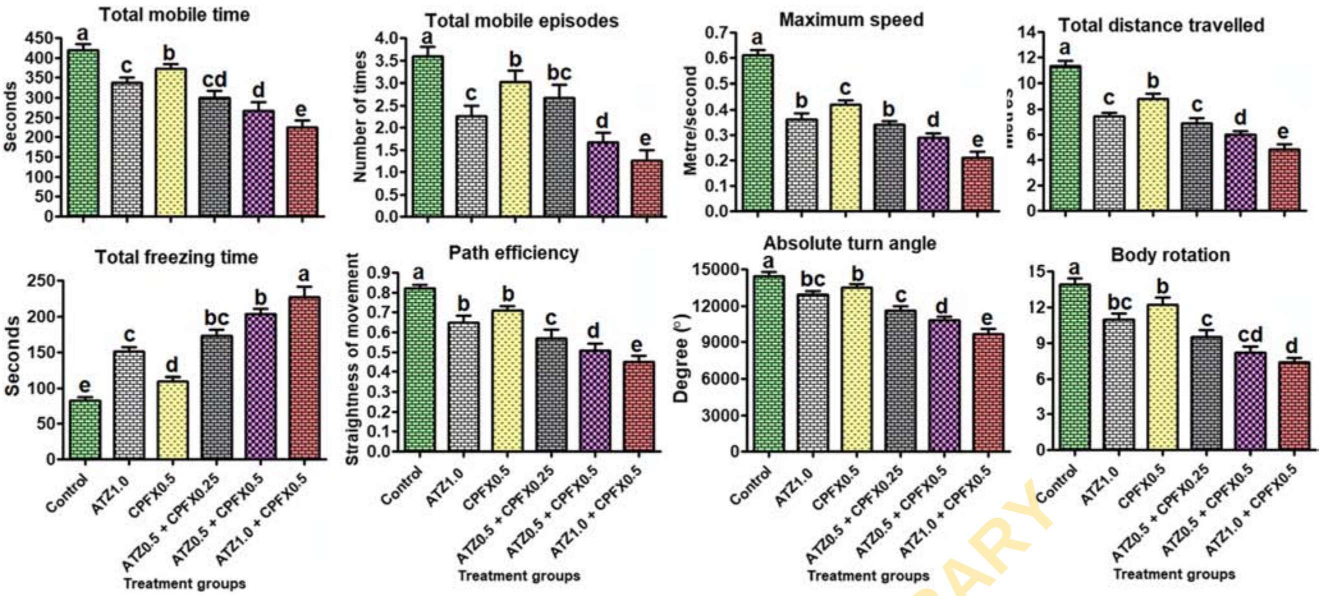


Fig. 1 Effects of individual and combined exposures to atrazine and ciprofloxacin on locomotor and motor activities in *N. cinerea*. Values represent mean ± SD. $n = 15$ insects. Columns with different letters above significantly differ

($F_{5,89} = 16.51$; $p < 0.0001$), and head $F_{5,89} = 27.93$; $p < 0.0001$) were significantly reduced in insects exposed to atrazine alone and ciprofloxacin alone when compared with control. The marked reductions in the AChE activity and GSH level were aggravated in insects co-exposed to their binary mixtures. In addition, separate exposure of insects to atrazine and ciprofloxacin significantly diminished activities of CAT (midgut $F_{5,89} = 154.6$, $p < 0.0001$; hemolymph $F_{5,89} = 46.68$, $p < 0.0001$; and head $F_{5,89} = 87.88$, $p < 0.0001$), GPx (midgut $F_{5,89} = 35.39$, $p < 0.0001$; hemolymph $F_{5,89} = 37.17$, $p < 0.0001$; and head $F_{5,89} = 122.2$, $p < 0.0001$), SOD (midgut $F_{5,89} = 27.68$, $p < 0.0001$; hemolymph $F_{5,89} = 30.78$, $p < 0.0001$; and head $F_{5,89} = 25.37$, $p < 0.0001$), and GST (midgut $F_{5,89} = 16.55$, $p < 0.0001$; hemolymph $F_{5,89} = 132.0$, $p < 0.0001$; and head $F_{5,89} = 16.88$, $p < 0.0001$) when compared with control. The diminutions in the activities of these antioxidant enzymes were exacerbated in insects co-exposed to their binary mixtures compared with individual exposure and control.

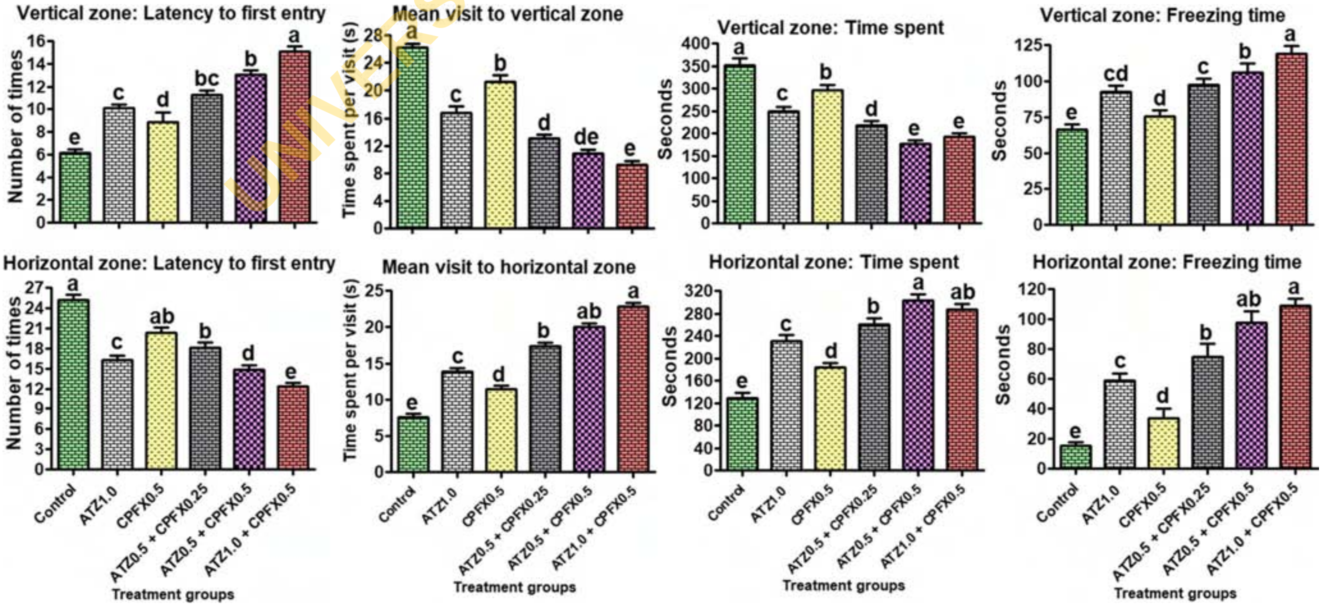


Fig. 2 Effects of separate and joint exposures to atrazine and ciprofloxacin on exploratory behaviors of *N. cinerea* in the vertical and horizontal parts of new apparatus. Values represent mean ± SD. $n = 15$ insects. Columns with different letters above significantly differ

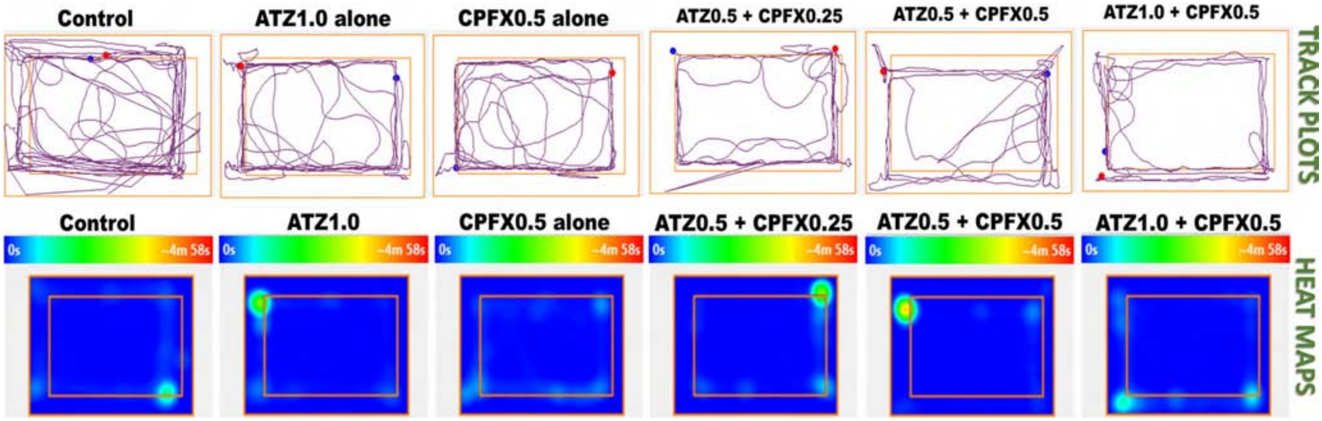
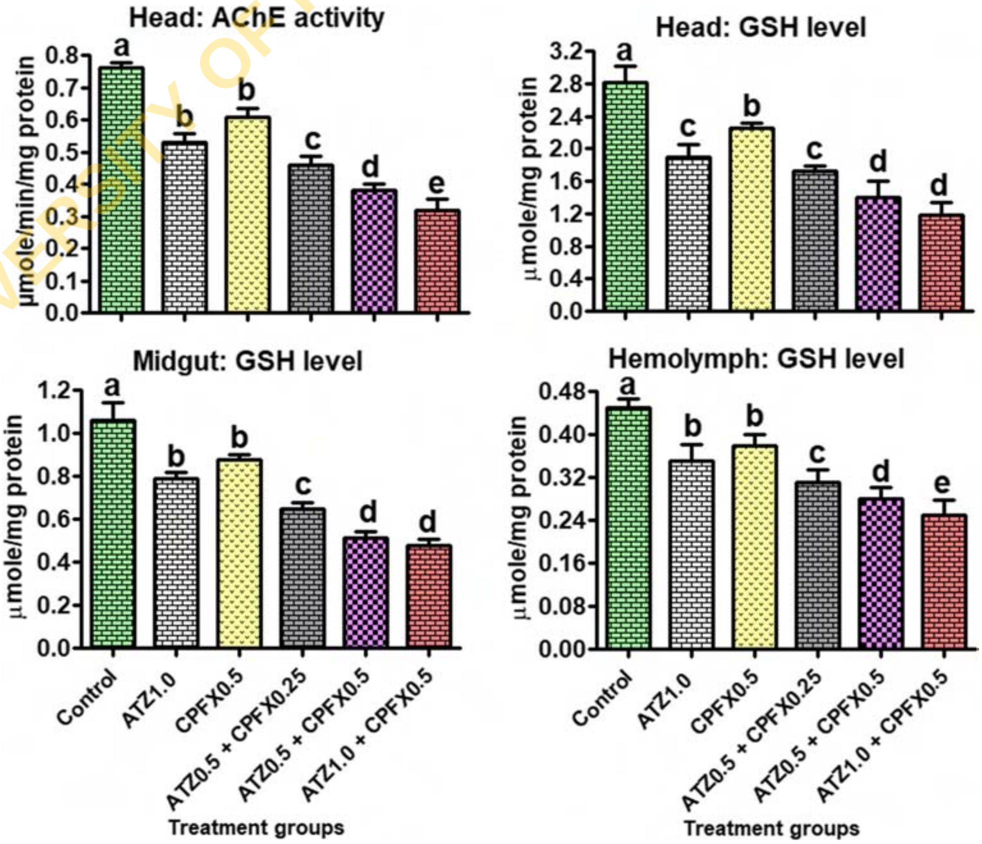


Fig. 3 Exploratory movements of *N. cinerea* described by track plots and heat maps. Each track plot illustrates the route covered by the insect while each heat map illustrates the light green spots that represent areas of intermittent motionlessness or home-base formation. Data were produced by video-tracking software (ANY-maze, Stoelting CO, USA)

Co-exposure to atrazine and ciprofloxacin heightens oxido-inflammatory stress response in *N. cinerea*

Furthermore, the levels of NO, an insects' cellular immunity mediator, as well as RONS and MDA were evaluated assessed to clarify the involvement of oxidative and inflammatory stress in the noxiousness resulting from exposure to the mixtures of atrazine and ciprofloxacin in *N. cinerea*. The NO, RONS, and LPO levels in the control and insects exposed to separate and binary mixtures of atrazine and ciprofloxacin are presented in Figs. 7 and 8. Exposure of insects to atrazine alone and ciprofloxacin alone significantly increased the levels of LPO (midgut $F_{5,89} = 151.4$, $p < 0.0001$; hemolymph F $_{5,89} = 67.48$, $p < 0.0001$; and head $F_{5,89} = 103.2$, $p < 0.0001$), NO (midgut $F_{5,89} = 136.1$, $p < 0.0001$; hemolymph $F_{5,89} = 110.4$, $p < 0.0001$; and head $F_{5,89} = 137.8$, $p < 0.0001$), and RONS (midgut $F_{5,89} = 152.1$, $p < 0.0001$; hemolymph F $_{5,89} = 88.63$, $p < 0.0001$; and head $F_{5,89} = 94.93$, $p < 0.0001$) when compared with control. The significant

Fig. 4 Effects of separate and joint exposures to atrazine and ciprofloxacin on the head AChE activity and GSH level in the midgut, hemolymph, and head of *N. cinerea* after 63 days. Values represent mean ± SD. *n* = 15 insects. Columns with different letters above significantly differ



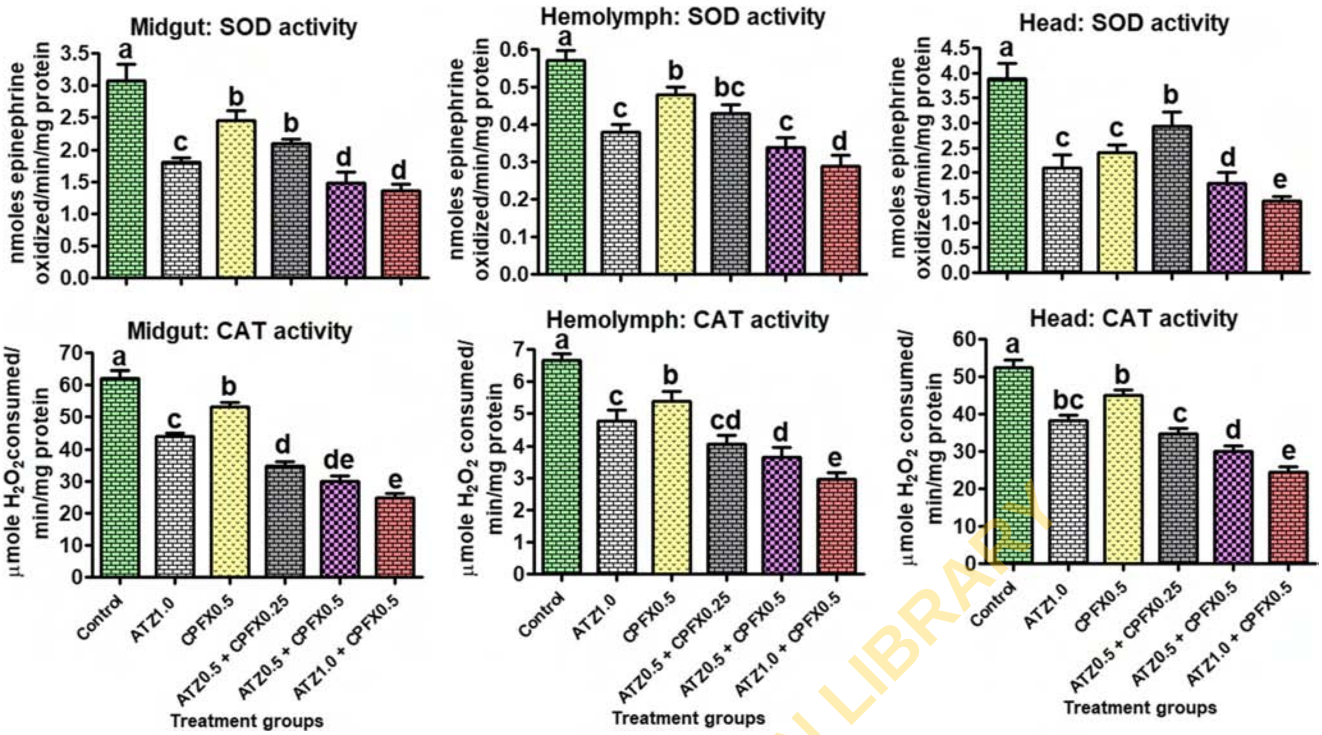


Fig. 5 Effects of separate and joint exposures to atrazine and ciprofloxacin on the SOD and CAT activities in the midgut, hemolymph, and head of *N. cinerea* after 63 days. Values represent mean ± SD. $n = 15$ insects. Columns with different letters above significantly differ

elevation in the NO, RONS, and LPO levels in the investigated tissues were significantly aggravated in the insects co-exposed to their binary mixtures when compared with individual exposure and control.

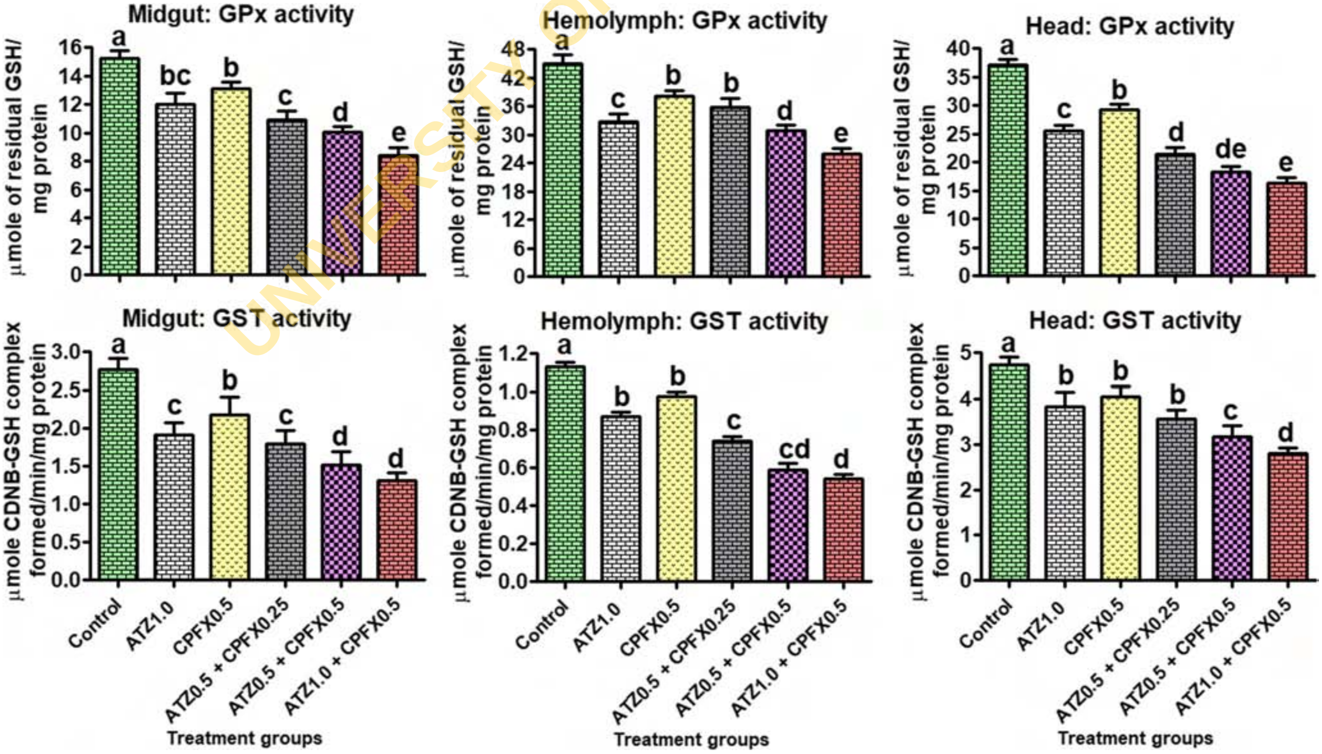


Fig. 6 Effects of separate and joint exposures to atrazine and ciprofloxacin on the GPx and GST activities in the midgut, hemolymph, and head of *N. cinerea* after 63 days. Values represent mean ± SD. $n = 15$ insects. Columns with different letters above significantly differ

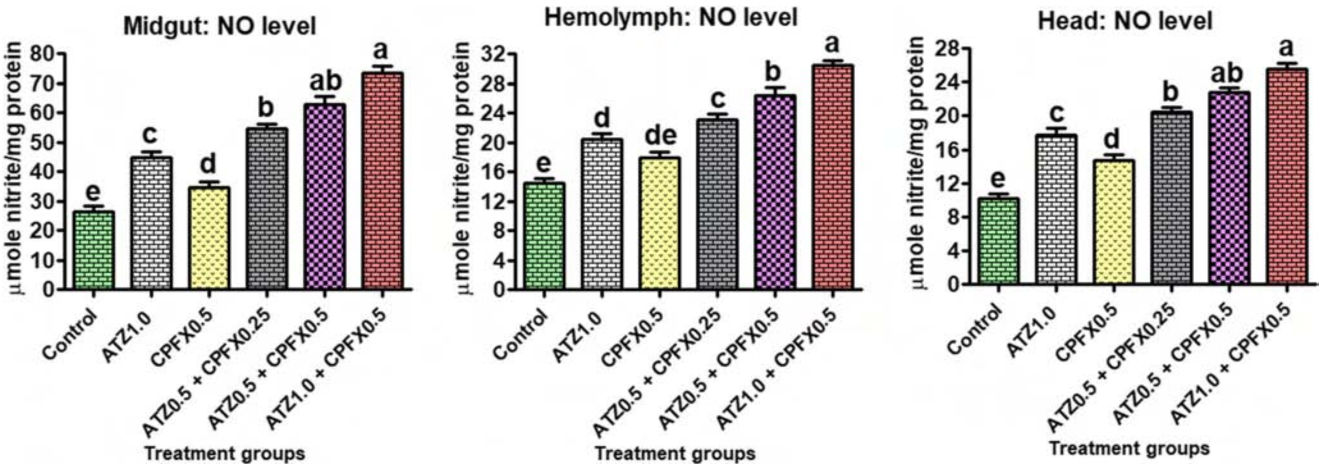


Fig. 7 Effects of separate and joint exposures to atrazine and ciprofloxacin on the NO level in the midgut, hemolymph, and head of *N. cinerea* after 63 days. Values represent mean ± SD. $n = 15$ insects. Columns with different letters above significantly differ

Discussion

Anthropogenic activities involving deliberate and inadvertent release of herbicide atrazine and pharmaceutical-containing wastewaters into different environmental compartments are internationally acknowledged to pose serious ecological risks (Sousa et al. 2018). Data from the present study shows, for first time, the ecotoxicological impact of separate and joint exposure to environmentally relevant concentrations of atrazine and ciprofloxacin in insects. Chronic exposure to individual and joint mixtures of atrazine and ciprofloxacin caused no adverse effect on the rate of survival and consumption of food, whereas it markedly affected locomotor actions and exploratory behavior of the exposed insects.

The marked reductions in the maximum speed, distance traveled, time and episodes of mobility with concomitant increase in the freezing time in insects separately exposed to atrazine and ciprofloxacin are consistent with locomotor deficits in exposed insects. Previous investigations demonstrated that exposure of mud snail *Potamopyrgus antipodarum* and Sprague-Dawley rats to atrazine significantly decreased their locomotor activities (Gerard and Poullain 2005; Rodriguez

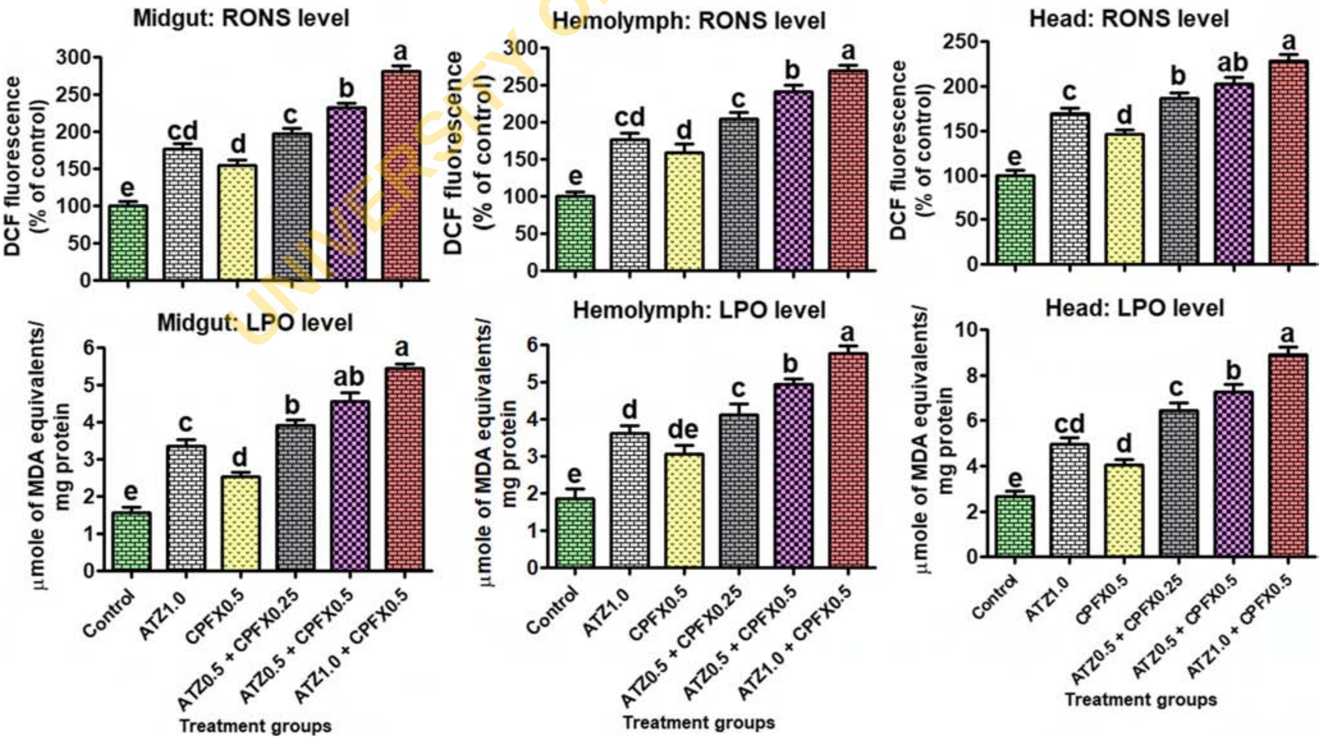


Fig. 8 Effects of separate and joint exposures to atrazine and ciprofloxacin on the RONS and LPO in the midgut, hemolymph, and head of *N. cinerea* after 63 days. Values represent mean ± SD. $n = 15$ insects. Columns with different letters above significantly differ

et al. 2017), whereas fluoroquinolones including ciprofloxacin decreased locomotion in rats and zebrafish (Thiel et al. 2001; Nogueira et al. 2019). The exacerbation in the locomotor dysfunctions in insects co-exposed to atrazine and ciprofloxacin reflects an additive noxious consequence of the binary mixtures on locomotor activities. Additionally, the reduction in the body rotation, turn angle, and straightness of movement in cockroaches treated with atrazine alone and ciprofloxacin alone indicates interference with turning and motor activities of exposed insects. Thus, the aggravation in the turning and motor deficits in cockroaches treated with the mixtures demonstrates an additive deleterious impact on muscular junctions and nervous system function in the insects.

The survival of organisms in the environment is tightly associated with its exploratory behavior to escape from predators and search of food (Adedara et al. 2020a). The decrease in exploratory activity in the vertical zone of the novel apparatus by insects singly exposed to atrazine and ciprofloxacin indicates altered behavioral or escape response. Emotional insects normally exhibit escape response by exploring the vertical zone while the less emotional explores the bottom zone of the new apparatus (Adedara et al. 2015). The decline in the mean visit and total time spent, as well as the increased freezing time and latency of entry to the periphery part in cockroaches separately exposed to atrazine and ciprofloxacin, connotes emotional modification, which was worsened in the binary mixtures groups. Moreover, the observation of greater heat map intensity and lesser track plots density in insects singly exposed to atrazine and ciprofloxacin corroborates impaired locomotor and behavioral deficits in the orientation and exploration of the insects. The altered exploratory deficiency in insects co-exposed to the binary mixtures of these contaminants may be of significant effect, as they may not escape predators, and consequently suffer decreased survival in their natural environment.

Several biochemical assays specifically AChE activity, GSH level, antioxidant enzyme activities, inflammatory, and oxidative stress biomarkers were performed using the supernatants of the midgut, hemolymph, and head of the exposed insects. In mechanistic terms, the regulation of locomotor and exploratory behaviors in insects is achieved by the cholinergic neurotransmission system (Malloy et al. 2019). Acetylcholine acts via nicotinic and muscarinic receptors to initiate cholinergic neurotransmission. The rapid hydrolysis of acetylcholine to acetate and choline by AChE normally terminates neurotransmission in the cholinergic synapses. Besides, AChE plays a pivotal role in the development and maintenance of synapses as well as neurite growth (Silman and Sussman 2005). Data from the present study showed that insects singly exposed to atrazine and ciprofloxacin exhibited reduced AChE activity which was exacerbated in insects co-exposed to their binary mixtures. Exposure to atrazine reportedly decreased AChE activity in chronically exposed insect damselfly *Coenagrion puella* (Campero et al. 2007) and in acutely exposed zebrafish (Liu et al. 2016). However, to our knowledge, this is the first report on the influence of ciprofloxacin on AChE activity in insects. Inhibition of AChE catalytic function may lead to excessive buildup of acetylcholine and, consequently, the down-regulation and desensitization of cholinergic receptors in the insects (van Koppen and Kaiser 2003; Adedara et al. 2020a). Thus, locomotor and exploratory deficits associated with atrazine and ciprofloxacin exposure may be caused by cholinergic dysfunction in the exposed insects.

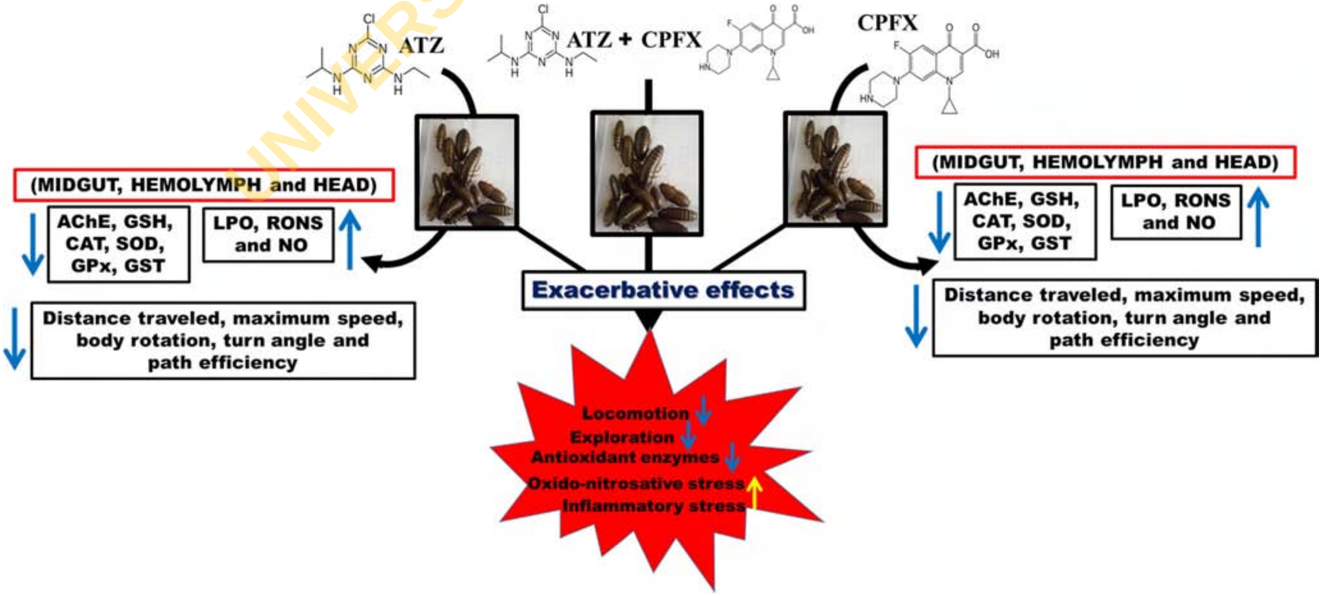


Fig. 9 Schematic illustration describing the effects of separate and combined exposures of *N. cinerea* to atrazine and ciprofloxacin. Exposure to the binary mixtures exacerbated the deleterious effects of atrazine alone and ciprofloxacin alone

Joint exposure to atrazine and ciprofloxacin amplified the oxidative stress condition induced by atrazine alone and ciprofloxacin alone in the midgut, hemolymph, and head of the exposed insects as evidenced by marked elevation in RONS and LPO levels cum diminution in antioxidant enzymes activities and GSH level in the current study. Cellular antioxidants are primarily responsible for the scavenging of free radicals before initiating oxidative and nitrosative stress mechanism leading to cellular damage (Poprac et al. 2017; Adedara et al. 2020b). The significant reduction in the CAT and SOD activities exhibited by insects singly and jointly exposed to atrazine and ciprofloxacin suggests a suppression of their antioxidant defense actions to neutralize cytotoxic radicals in the midgut, hemolymph, and head of the exposed insects. In addition, the significant depletion in the level of GSH, a principal non-enzymatic antioxidant, which also acts as substrate for GSH-dependent enzymes (GPx and GST) may result from its excessive demand, and consequently, the reduction in the GPx and GST activities in the midgut, hemolymph, and head in exposed insects.

Oxidative devastation of cellular polyunsaturated lipid component by free radicals to yield is MDA and 4-hydroxynonenal as final products is referred to as lipid peroxidation (LPO). In the current investigation, the decrease in endogenous enzymatic and non-enzymatic antioxidants caused membrane destabilization and induction of oxidative injury in the midgut, hemolymph, and head of insects singly exposed to atrazine and ciprofloxacin as evidenced by elevated RONS and LPO levels. Moreover, low intracellular concentration of NO molecules is indispensable for nervous system function and immunity in insects (Müller 1997; Sadekuzzaman et al. 2018). However, NO is a well-known inflammatory mediator, and its uncontrolled production may induce nitrosative stress and cause lipid, protein, and nucleic acids modification, subsequent to exhaustion of cellular antioxidant defense mechanisms. The current data showed that the joint exposure to binary mixtures of atrazine and ciprofloxacin posed greater nitrosative, inflammatory, and oxidative stress threat compared to insects singly exposed to atrazine or ciprofloxacin. The schematic illustration describing effects of separate and combined exposures of insects to atrazine and ciprofloxacin is represented in Fig. 9.

Conclusions

Combined exposure to ciprofloxacin and atrazine at ecologically relevant concentrations elicited greater locomotor and exploratory deficits than each individual compound in non-target insect *N. cinerea*. In addition, AChE activity, lipid peroxidation, RONS, and antioxidant enzyme activities are valuable indices for the evaluation of toxicity resulting from exposure to anthropogenic contaminants in insects. The novel data reported herein further establish the utility *N. cinerea* as an invaluable experimental model insect for addressing ecological risks of contaminants to non-target insect species.

Authors contributions a) Isaac A. Adedara: conceptualization; supervision; methodology; writing - original draft preparation, review, and editing.

b) Umin-Awaji S. Godswill: methodology; data curation; software.

c) Miriam A. Mike: methodology; data curation; software.

d) Blessing A. Afolabi: conceptualization; methodology; software, writing - original draft preparation.

e) Chizoba C. Amorha: methodology; data curation; software.

f) Joseph Sule: methodology; data curation; software.

g) Joao B. T. Rocha: conceptualization; validation; writing – review and editing.

h) Ebenezer O. Farombi: conceptualization; validation; writing – review and editing.

Data availability The original data and materials of the current study are available with the corresponding author and would be made available on justifiable request.

Compliance with ethical standards

Ethics approval Not applicable. *N. cinerea* is an invertebrate model organism (insect) which requires no ethics approval.

Consent to participate Not applicable. This is an animal study which does not require consent to participate.

Consent to publish The content of this manuscript is original. It does not contain data and pictures of any person. Hence, no consent from any person or organization is required to publish it.

Competing interests The authors declare that there are no conflicts of interest.

References

Abdulelah SA, Crile KG, Almouseli A, Awali S, Tutwiler AY, Tien EA, Manzo VJ, Hadeed MN, Belanger RM (2020) Environmentally relevant atrazine exposures cause DNA damage in cells of the lateral antennules of crayfish (*Faxonius virilis*). Chemosphere 239:124786. https://doi.org/10.1016/j.chemosphere.2019.124786

Adedara IA, Rosemberg DB, Souza DO, Kamdem JP, Farombi EO, Aschner M, Rocha JBT (2015) Biochemical and behavioral deficits in lobster cockroach *Nauphoeta cinerea* model of methylmercury exposure. Toxicol Res 4:442–451

Adedara IA, Rosemberg DB, de Souza D, Farombi EO, Aschner M, Souza DO, Rocha JBT (2016) Neurobehavioral and biochemical changes in *Nauphoeta cinerea* following dietary exposure to chlorpyrifos. Pestic Biochem Physiol 130:22–30

Adedara IA, Abolaji AO, Awogbindin IO, Farombi EO (2017) Suppression of the brain-pituitary-testicular axis function following acute arsenic and manganese co-exposure and withdrawal in rats. J Trace Elem Med Biol 39:21–29

Adedara IA, Awogbindin IO, Owoeye O, Maduako IC, Ajeleti AO, Owumi SE, Patlolla AK, Farombi EO (2020b) Kolaviron via anti-inflammatory and redox regulatory mechanisms abates multi-walled carbon nanotubes-induced neurobehavioral deficits in rats. Psychopharmacology 237:1027–1040

Adedara IA, Ajayi BO, Afolabi BA, Awogbindin IO, Rocha JBT, Farombi EO (2021) Toxicological outcome of exposure to psychoactive drugs carbamazepine and diazepam on non-target insect *Nauphoeta cinerea*. Chemosphere 264:128449

Adedara IA, Awogbindin IO, Afolabi BA, Ajayi BO, Rocha JBT, Farombi EO (2020a) Hazardous impact of diclofenac exposure on the behavior and antioxidant defense system in *Nauphoeta cinerea*. Environ Pollut 265:115053. https://doi.org/10.1016/j.envpol.2020.115053

Aebi H (1984) Catalase *in vitro*. Methods Enzymol 105:121–126

Afolabi BA, Olagoke OC, Souza DO, Aschner M, Rocha JBT, Segatto ALA (2020) Modified expression of antioxidant genes in lobster cockroach, *Nauphoeta cinerea* exposed to methylmercury and monosodium glutamate. Chem Biol Interact 318:108969. https://doi.org/10.1016/j.cbi.2020.108969

Arun S, Kumar RM, Ruppa J, Mukhopadhyay M, Ilango K, Chakraborty P (2020) Occurrence, sources and risk assessment of fluoroquinolones in dumpsite soil and sewage sludge from Chennai, India. Environ Toxicol Pharmacol 79:103410. https://doi.org/10.1016/j.etap.2020.103410

Asouzu Johnson J, Ihunwo A, Chimuka L, Mbajiorgu EF (2019) Cardiotoxicity in African clawed frog (*Xenopus laevis*) subchronically exposed to environmentally relevant atrazine concentrations: implications for species survival. Aquat Toxicol 213:105218

Bell AT, Niven JE (2016) Strength of forelimb lateralization predicts motor errors in an insect. Biol Lett 12:20160547

Bell WJ, Roth LM, Nalepa CA (2007) Cockroaches: ecology, behavior, and natural history. The Johns Hopkins University Press

Blahova J, Dobsikova R, Enevova V, Modra H, Plhalova L, Hostovsky M, Marsalek P, Mares J, Skoric M, Vecerek V, Svobodova Z (2020) Comprehensive fitness evaluation of common carp (*Cyprinus carpio* L.) after twelve weeks of atrazine exposure. Sci Total Environ 718:135059. https://doi.org/10.1016/j.scitotenv.2019.135059

Blankenburg S, Balfanz S, Hayashi Y, Shigenobu S, Miura T, Baumann O, Baumann A, Blenau W (2015) Cockroach GABAB receptor subtypes: molecular characterization, pharmacological properties and tissue distribution. Neuropharmacology 88:134–144

Bondarczuk K, Markowicz A, Piotrowska-Seget Z (2016) The urgent need for risk assessment on the antibiotic resistance spread via sewage sludge land application. Environ Int 87:49–55

Bradford MM (1976) Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Campero M, Ollevier F, Stoks R (2007) Ecological relevance and sensitivity depending on the exposure time for two biomarkers. Environ Toxicol 22:572–581

Cheng Y, Zhu L, Song W, Jiang C, Li B, Du Z, Wang J, Wang J, Li D, Zhang K (2020) Combined effects of mulch film-derived microplastics and atrazine on oxidative stress and gene expression in earthworm (*Eisenia fetida*). Sci Total Environ 746:141280. https://doi.org/10.1016/j.scitotenv.2020.141280

St Clair CR, Fuller CA (2018) Atrazine exposure influences immunity in the blue dasher dragonfly, *Pachydiplax longipennis* (Odonata: Libellulidae). J Insect Sci 18:12. https://doi.org/10.1093/jisesa/iey095

Dou R, Sun J, Deng F, Wang P, Zhou H, Wei Z, Chen M, He Z, Lai M, Ye T, Zhu L (2020) Contamination of pyrethroids and atrazine in greenhouse and open-field agricultural soils in China. Sci Total Environ 701:134916

Ellman GL, Courtney KD, Andres V Jr, Feather-Stone RM (1961) A new and rapidcolorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7:88–95

García-Espiñeira M, Tejeda-Benitez L, Olivero-Verbel J (2018) Toxicity of atrazine- and glyphosate-based formulations on *Caenorhabditis elegans*. Ecotoxicol Environ Saf 156:216–222

Gerard C, Poullain V (2005) Variation in the response of the invasive species *Potamopyrgus antipodarum* (Smith) to natural (cyanobacterial toxin) and anthropogenic (herbicide atrazine) stressors. Environ Pollut 138:28–33

Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR (1982) Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. Anal Biochem 126:131–138

Griboff J, Morales D, Bertrand L, Bonansea RI, Monferrán MV, Asis R, Wunderlin DA, Amé MV (2014) Oxidative stress response induced by atrazine in *Palaemonetes argentinus*: the protective effect of vitamin E. Ecotoxicol Environ Saf 108:1–8

Groten JP, Feron VJ, Sühnel J (2001) Toxicology of simple and complex mixtures. Trends Pharmacol Sci 22:316–322

Habig WH, Pabst MJ, Jakoby WB (1974) Glutathione S-transferase. The first enzymatic step in mercapturic acid formation. J Biol Chem 249:7130–7139

Harris WE, Moore PJ (2005) Sperm competition and male ejaculate investment in *Nauphoeta cinerea*: effects of social environment during development. J Evol Biol 18:474–480

Ilgin S, Can OD, Atli O, Ucel UI, Sener E, Guven I (2015) Ciprofloxacin-induced neurotoxicity: evaluation of possible underlying mechanisms. Toxicol Mech Methods 25:374–381

Jollow DJ, Mitchell JR, Zampaglione N, Gillette JR (1974) Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. Pharmacology 11:151–169

Komtchou S, Dirany A, Drogui P, Robert D, Lafrance P (2017) Removal of atrazine and its by-products from water using electrochemical advanced oxidation processes. Water Res 125:91–103

Li C, Chen J, Wang J, Ma Z, Han P, Luan Y, Lu A (2015) Occurrence of antibiotics in soils and manures from greenhouse vegetable production bases of Beijing, China and an associated risk assessment. Sci Total Environ 521–522:101–107

Liu JL, Wong MH (2013) Pharmaceuticals and personal care products (PPCPs): a review on environmental contamination in China. Environ Int 59:208–224

Liu Z, Wang Y, Zhu Z, Yang E, Feng X, Fu Z, Jin Y (2016) Atrazine and its main metabolites alter the locomotor activity of larval zebrafish (*Danio rerio*). Chemosphere 148:163–170

Liu J, Li X, Wang X (2019) Toxicological effects of ciprofloxacin exposure to *Drosophila melanogaster*. Chemosphere 237:124542. https://doi.org/10.1016/j.chemosphere.2019.124542

Malloy CA, Somasundaram E, Omar A, Bhutto U, Medley M, Dzubuk N, Cooper RL (2019) Pharmacological identification of cholinergic receptor subtypes: modulation of locomotion and neural circuit excitability in Drosophila larvae. Neuroscience 411:47–64

Marcus SR, Fiumera AC (2016) Atrazine exposure affects longevity, development time and body size in Drosophila melanogaster. J Insect Physiol 91–92:18–25

Misra HP, Fridovich I (1972) The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 247:3170–3175

Mrdaković M, Ilijin L, Vlahović M, Filipović A, Grčić A, Todorović D, Perić-Mataruga V (2019) Effects of dietary fluoranthene on nymphs of *Blaptica dubia* S. (Blattodea: Blaberidae). Environ Sci Pollut Res Int 26:6216–6222

Müller U (1997) The nitric oxide system in insects. Prog Neurobiol 51:363–381

Nogueira AF, Pinto G, Correia B, Nunes B (2019) Embryonic development, locomotor behavior, biochemical, and epigenetic effects of the pharmaceutical drugs paracetamol and ciprofloxacin in larvae and embryos of *Danio rerio* when exposed to environmental realistic levels of both drugs. Environ Toxicol 34:1177–1190

Östman M, Lindberg RH, Fick J, Björn E, Tysklind M (2017) Screening of biocides, metals and antibiotics in Swedish sewage sludge and wastewater. Water Res 115:318–328

Poprac P, Jomova K, Simunkova M, Kollar V, Rhodes CJ, Valko M (2017) Targeting free radicals in oxidative stress-related human diseases. Trends Pharmacol Sci 38:592–607

Rodrigues NR, Nunes ME, Silva DG, Zemolin AP, Meinerz DF, Cruz LC, Pereira AB, Rocha JB, Posser T, Franco JL (2013) Is the lobster cockroach *Nauphoeta cinerea* a valuable model for evaluating mercury induced oxidative stress? Chemosphere 92:1177–1182

Rodriguez VM, Mendoza-Trejo MS, Hernandez-Plata I, Giordano M (2017) Behavioral effects and neuroanatomical targets of acute atrazine exposure in the male Sprague-Dawley rat. Neurotoxicology 58: 161–170

Romano D, Benelli G, Kavallieratos NG, Athanassiou CG, Canale A, Stefanini C (2020) Beetle-robot hybrid interaction: sex, lateralization and mating experience modulate behavioural responses to robotic cues in the larger grain borer *Prostephanus truncatus* (Horn). Biol Cybernetics 114:473–483

Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG (1973) Selenium: biochemical role as a component of glutathione peroxidase. Science 179:588–590

Saalfeld GQ, Varela Junior AS, Castro T, Pereira FA, Gheller SMM, da Silva AC, Corcini CD, da Rosa CE, Colares EP (2018) Low atrazine dosages reduce sperm quality of *Calomys laucha* mice. Environ Sci Pollut Res Int 25:2924–2931

Sadekuzzaman M, Stanley D, Kim Y (2018) Nitric oxide mediates insect cellular immunity via phospholipase A2 activation. J Innate Immun 10:70–81

Semren TŽ, Žunec S, Pizent A (2018) Oxidative stress in triazine pesticide toxicity: a review of the main biomarker findings. Arh Hig Rada Toksikol 69:109–125

Silman I, Sussman JL (2005) Acetylcholinesterase: 'classical' and 'non-classical' functions and pharmacology. Curr Opin Pharmacol 5: 293–302

Sisay M, Weldegebreal F, Tesfa T, Ataro Z, Marami D, Mitiku H, Motbaynor B, Teklemariam Z (2018) Resistance profile of clinically relevant bacterial isolates against fluoroquinolone in Ethiopia: a systematic review and meta-analysis. BMC Pharmacol Toxicol 19:86. https://doi.org/10.1186/s40360-018-0274-6

Song Y, Zhu LS, Xie H, Wang J, Wang JH, Liu W, Dong XL (2009) Effects of atrazine on DNA damage and antioxidative enzymes in Vicia faba. Environ Toxicol Chem 28:1059–1062

Sousa JCG, Ribeiro AR, Barbosa MO, Pereira MFR, Silva AMT (2018) A review on environmental monitoring of water organic pollutants identified by EU guidelines. J Hazard Mater 344:146–162

Stankiewicz M, Dąbrowski M, de Lima ME., 2012. Nervous system of *Periplaneta americana* cockroach as a model in toxinological studies: a short historical and actual view. J Toxicol 2012:143740. https://doi.org/10.1155/2012/143740, 1, 11

Stara A, Kouba A, Velisek J (2018) Biochemical and histological effects of sub-chronic exposure to atrazine in crayfish Cherax destructor. Chem Biol Interact 291:95–102

Stürmer GD, de Freitas TC, Heberle Mde A, de Assis DR, Vinadé L, Pereira AB, Franco JL, Dal Belo CA (2014) Modulation of dopaminergic neurotransmission induced by sublethal doses of the organophosphate trichlorfon in cockroaches. Ecotoxicol Environ Saf 109:56–62

Thiel R, Metzner S, Gericke C, Rahm U, Stahlmann R (2001) Effects of fluoroquinolones on the locomotor activity in rats. Arch Toxicol 75: 36–41

van Koppen CJ, Kaiser B (2003) Regulation of muscarinic acetylcholine receptor signaling. Pharmacol Ther 98:197–220

Waczuk EP, Wagner R, Klein B, da Rocha JBT, Ardisson-Araújo DMP, Barbosa NV (2019) Assessing the toxicant effect of spontaneously volatilized 4-vinylcyclohexane exposure in nymphs of the lobster cockroach *Nauphoeta cinerea*. Environ Toxicol Pharmacol 72: 103264

Wang S, Zhang Q, Zheng S, Chen M, Zhao F, Xu S (2019) Atrazine exposure triggers common carp neutrophil apoptosis via the CYP450s/ROS pathway. Fish Shellfish Immunol 84:551–557

Wei R, He T, Zhang S, Zhu L, Shang B, Li Z, Wang R (2019) Occurrence of seventeen veterinary antibiotics and resistant bacterias in manure-fertilized vegetable farm soil in four provinces of China. Chemosphere 215:234–240

Yang X, Li Y, Wang X (2020) Effects of ciprofloxacin exposure on the earthworm *Eisenia fetida*. Environ Pollut 262:114287. https://doi.org/10.1016/j.envpol.2020.114287

Zhang H, Zhou Y, Huang Y, Wu L, Liu X, Luo Y (2016) Residues and risks of veterinary antibiotics in protected vegetable soils following application of different manures. Chemosphere 152:229–237

Zhu LS, Shao B, Song Y, Xie H, Wang J, Wang JH, Liu W, Hou XX (2011) DNA damage and effects on antioxidative enzymes in zebra fish (Danio rerio) induced by atrazine. Toxicol Mech Methods 21: 31–36